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<p>(54) Title: PROSTAGLANDIN DERIVATIVES DEVOID OF SIDE-EFFECTS FOR THE TREATMENT OF GLAUCOMA</p> <p>(57) Abstract</p> <p>A new method and compositions for the treatment of glaucoma and ocular hypertension are described. The method is based on the usage of EP₁ prostanoid receptor agonists which effectively reduce the intraocular pressure but have no, or reduced effect on iris pigmentation. The prostaglandin analogue which is an EP₁ selective agonist is applied topically on the eye.</p>			

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PROSTAGLANDIN DERIVATIVES DEVOID OF SIDE-EFFECTS FOR THE TREATMENT OF GLAUCOMA

The invention is related to a method of treating glaucoma and ocular hypertension utilizing prostaglandin analogues or derivatives that are devoid of, or have reduced melanogenic effect in the eye. The invention also relates to ophthalmic compositions containing prostaglandin compounds devoid of, or have reduced melanogenic effect in the eye.

Glaucoma is an eye disorder characterized by increased intraocular pressure, excavation of the optic nerve head, and gradual loss of the visual field. An abnormally high intraocular pressure is commonly known to be detrimental to the eye, and there are clear indications that in glaucoma the intraocular pressure is the most important factor causing degenerative changes in the retina and the optic nerve head. The exact pathophysiological mechanism of open angle glaucoma is, however, still unknown. Unless treated, glaucoma may lead to blindness, the course of the disease typically being slow with progressive loss of vision.

The intraocular pressure (IOP) can be defined according to the formula (1):

$$(1) \quad IOP = Pe + (Ft - Fu) \times R$$

where Pe is the episcleral venous pressure, Ft the formation of aqueous humor, Fu the part of the aqueous humor which exits the eye through the uveoscleral outflow pathway, and R is the resistance in the trabecular outflow pathway. The aqueous humor in the anterior and posterior chambers of the eye is produced by the ciliary processes behind the iris. It then flows through the pupil into the anterior chamber, and normally exits the eye through the trabecular meshwork and Schlemm's canal into the episcleral veins outside the eye globe. However, part of the aqueous humor may leave the eye through the uveoscleral outflow route. The flow in this route is regarded as only minimally influenced by the intraocular pressure (Bill, 1975).

The intraocular pressure in humans is normally in the range of 12-22 mmHg. At higher pressures, e.g. above 22 mmHg, there is an increased risk that the eye may be

damaged. In one particular form of glaucoma, normal tension glaucoma, damage may, however, occur at intraocular pressure levels that are within the normal physiological range. The opposite situation is also known, i.e. some individuals may exhibit an abnormally high intraocular pressure without any manifest defects in the visual field or the optic nerve head. Such conditions usually are referred to as ocular hypertension.

Glaucoma treatment can be given by means of drugs, laser or surgery. In drug treatment the purpose is to reduce either the formation of aqueous humor (F_t), or the resistance to outflow of aqueous humor (R), which according to formula (1) above will result in reduced intraocular pressure; alternatively to increase the outflow of aqueous humor through the uveoscleral route which according to formula (1) also reduces the intraocular pressure.

Prostaglandins and typically PGF_{2α}, its esters and analogues, reduce the intraocular pressure mainly by increasing the uveoscleral outflow of aqueous humor (Crawford and Kaufman, 1987; Nilsson et al., 1989; Toris et al., 1993; Stjernschantz et al., 1995). The use of prostaglandins and their derivatives is described in several patents and patent applications, for instance in US 4,599,353 (Bito), US 4,952,581 (Bito), WO89/03384 (Resul and Stjernschantz), EP 170258 (Cooper), EP 253094 (Goh), and in EP 308135 (Ueno).

Prostaglandins are fatty acids usually derived from the precursors eicosatrienoic, eicosatetraenoic and eicosapentaneoic acid through metabolic steps involving oxygenation. Naturally occurring prostaglandins typically have the general structure shown in Fig. 1.

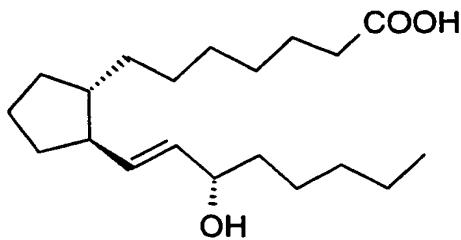


Fig. 1

The prostaglandins accordingly carry a cyclopentane ring to which two carbon chains link, the upper chain usually being called the alpha chain and the lower chain usually being called the omega chain. The prostaglandins are classified in subgroups A, B, C, D, E, F, G, H, I and J depending on the structure and substituents in the cyclopentane ring as shown in Fig. 2.

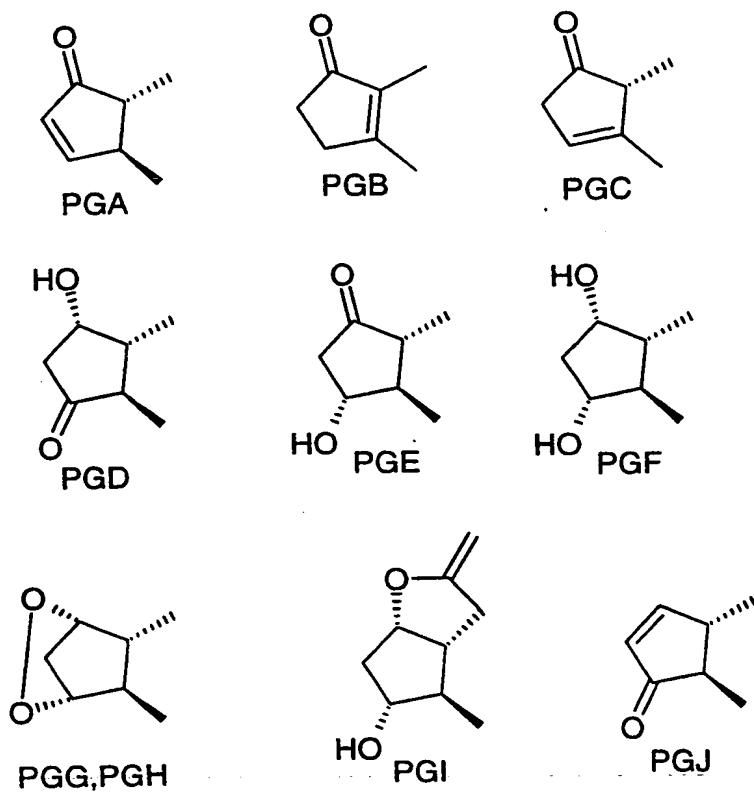


Fig. 2

The alpha chain is a 7 carbon carboxy-terminated aliphatic chain whereas the omega chain is an 8 carbon methyl-terminated aliphatic chain. Depending on the number of double bonds in these chains subscripts of 1 to 3 are given. In prostaglandins with subscript 1, e.g. PGF_{1α}, the double bond is situated between carbons 13 and 14 in the omega chain, and it exhibits trans configuration in naturally occurring prostaglandins. In prostaglandins with subscript 2, e.g. PGF_{2α}, an additional double bond in the cis configuration is situated between carbons 5 and 6 in the alpha chain, and finally in

prostaglandins with subscript 3, a third double bond is situated between carbons 17 and 18 in the omega chain. This double bond also exhibits cis configuration in naturally occurring prostaglandins. All naturally occurring prostaglandins carry a hydroxyl group on carbon 15, which is essential for biological activity.

The receptor system for the naturally occurring prostaglandins has only recently been elucidated. Thus most of the prostaglandin receptors have been pharmacologically characterized and their molecular structure identified by molecular biological techniques (Coleman et al., 1994). There are specific receptors for the naturally occurring prostaglandins. The receptors for PGD, PGE, PGF, PGI₂ (prostacyclin) and TXA₂ (thromboxane) being abbreviated DP, EP, FP, IP, and TP, respectively. Furthermore it has been shown that the EP receptor can be subdivided into four receptors, namely the EP₁, EP₂, EP₃, and EP₄ receptors. Specific tissues or cells may express only a few or many of these receptors, depending on the evolutionary development of this autacoid system in different species. Thus for instance it has been shown that the cat iris sphincter muscle expresses predominantly FP receptors that are functionally coupled and mediate constriction of the pupil, while the corresponding smooth muscle of the bovine eye expresses EP₁ and TP receptors, activation of either of which will result in contraction of the muscle. A compound which binds to a specific receptor and activates it is called an agonist while a compound that only binds to a receptor without activating it is called an antagonist.

With respect to the practical usefulness of many of the prostaglandins and their derivatives as suitable drugs for treatment of glaucoma or ocular hypertension, a limiting factor may be their property of causing increased pigmentation of the iris in the eye (Stjernschantz and Alm, 1996). Thus, the colour of the iris during chronic treatment in monkeys and in man tends to become darker, turning into brown. While this apparently has no negative medical consequences, it is a clear disadvantage from a cosmetic point of view, particularly in patients undergoing treatment only in one eye. It would thus be desirable to identify prostaglandins which effectively reduce the intraocular pressure without causing the side-effect of increased iridial pigmentation.

We have now unexpectedly found that prostaglandin derivatives and analogues which are selective agonists for the EP₁ subgroup of prostanoid receptors fulfil the criteria for a prostaglandin analogue which effectively reduces the intraocular pressure without causing increased production of pigment (melanogenesis) in the iris. The background of this finding is that in a study to identify the prostanoid receptor subtypes in the human iridial melanocytes we have found that these cells express in their cell membranes the FP, EP₂, and EP₃ receptors, but not the EP₁ and TP receptors. Furthermore, we have investigated the intraocular pressure reducing effect of several relatively selective EP₁ agonists and found that these prostaglandin analogues effectively and potently reduce the intraocular pressure both in cats and monkeys.

Therefore, it is now apparent that, by using selective EP₁ receptor agonists, the intraocular pressure can be reduced in primates, and thus also in man, without increased, or with significantly reduced melanogenesis as side-effect, since the melanin producing cells, the melanocytes, lack the specific receptor necessary for the transmembrane signalling into the cell. While we at present have no clinical evidence that such selective EP₁ agonists do not cause increased pigmentation of the iris, since the induction time for this phenomenon to occur often is 6-12 months, and thus extremely long and costly experiments have to be carried out in primates, we can nevertheless conclude from relevant *in vitro* studies that such increased pigmentation would not occur in the absence of the specific signalling receptor in the cell membrane of the melanocytes.

Accordingly, high selectivity or specificity to the EP₁ receptor compared to other prostaglandin receptors in the eye characterizes the compounds to be used in the method or compositions according to the present invention. It need not to be said that the more selective the compound is for the EP₁ receptor, the better results are obtained but a certain advantage is of course achieved also in cases of some interaction with other receptors. High selectivity in this connection means that the effect on the EP₁ receptor is at least more than 5 times, especially more than 10 times, and in particular more than 100 or 1000 times the effect on the other prostaglandin receptors.

The specific prostaglandin analogues that we have used for exemplifying and proving this invention were PGF_{2β} (1), PGF_{2β} isopropyl ester (2), 17-phenyl-18,19,20-trinor-PGE₂ (3), 17-phenyl-18,19,20-trinor-PGE₂ isopropyl ester (4), 15(R,S)-16,16-trimethylene-PGE₂ (5), 15(R,S)-16,16-trimethylene-PGE₂ methyl ester (6), and 13,14-dihydro-17-(3-fluorophenyl)-18,19,20 trinor-PGE₂-isopropyl ester (7). All these analogues are relatively selective EP₁ receptor agonists. The receptor profiles of the test compounds are presented in Table I.

Table I. Receptor profile of prostaglandin analogues tested (EC-50 values expressed as moles/l in functional receptor assays).

Prostaglandin	FP	EP ₁	EP ₂	EP ₃	DP/IP	TP
1 >10 ⁻³	5x10 ⁻⁶	10 ⁻⁶	10 ⁻⁵	>10 ⁻⁴	>10 ⁻³	
3 >10 ⁻⁴	10 ⁻⁷	2x10 ⁻⁸	>10 ⁻⁴	>10 ⁻⁴	>10 ⁻⁴	
5	2x10 ⁻⁵	6x10 ⁻⁹	2x10 ⁻⁷	3x10 ⁻⁸ #	>10 ⁻⁴	>10 ⁻⁴
7	6x10 ⁻⁷	4x10 ⁻⁸	5x10 ⁻⁵	10 ⁻⁶ #	>10 ⁻⁴	>10 ⁻⁴

estimated based on difference in receptor assay between guinea pig vas deferens and chick ileum.

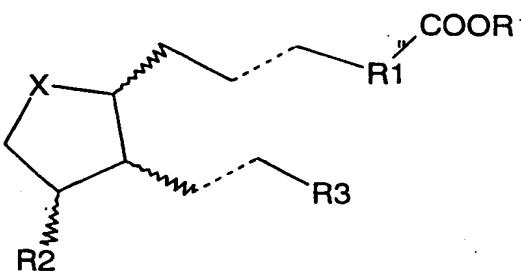
In one aspect, the invention relates to the use of selective prostaglandin EP₁ receptor agonists devoid of melanogenic effect for the treatment of glaucoma or ocular hypertension. The method for treating glaucoma or ocular hypertension comprises contacting the surface of the eye with an effective intraocular pressure reducing amount of a composition, containing an EP₁ selective prostaglandin as aforesaid, in order to reduce the intraocular pressure and to maintain said pressure at a reduced level. The composition usually contains about 0.1-100 µg, especially 1-30 µg per application of the active substance. The composition is applied topically on the eye 1-3 times daily.

The prostaglandin derivative is mixed with an ophthalmologically compatible vehicle known per se. The vehicle which may be employed for preparing compositions of this invention comprises aqueous solutions, e.g. physiological saline, oil solutions, or ointments. The vehicle may furthermore contain ophthalmologically compatible preservatives such as e.g. benzalkonium chloride, surfactants, such as polysorbate 80, liposomes or polymers, for example methyl cellulose, polyvinyl alcohol, polyvinyl pyrrolidone and hyaluronic acid; these may be used for increasing the viscosity. Furthermore it is also possible to use soluble or insoluble drug inserts.

In another aspect, the invention relates to ophthalmological compositions for medical treatment of glaucoma or ocular hypertension which comprise an effective intraocular pressure reducing amount of a prostaglandin analogue which is a selective agonist of EP₁ receptors as defined above and an ophthalmologically compatible carrier. The effective amount usually comprises a dose of about 0.1-100 µg in about 10-50 µl of the composition. The compositions according to the present invention are clear improvements over the prior art prostaglandin compositions due to the selectivity of the active compound for EP₁ receptors compared to other prostaglandin receptors with the risk for pigmentation eliminated or at least substantially reduced.

In still another aspect, the invention relates to the use of the prostaglandin analogue for the preparation of a medicament for treatment of glaucoma and ocular hypertension.

Preferably, the prostaglandin analogue is derived from PGF or PGE type prostaglandins. Particularly, the prostaglandin analogue is a compound of the general formula:



wherein:

the wavy bonds represent the α or β configuration, and the dashed bonds represent a single bond, a triple bond or a double bond in the cis or trans configuration;

R is hydrogen, saturated or unsaturated alkyl, preferably C₁₋₁₀ alkyl, cycloalkyl, preferably C₃₋₈ cycloalkyl, aryl, arylalkyl, preferably aryl-C₂₋₅ alkyl, or heteroaryl;

R1 is a saturated or unsaturated alkyl group having 2-5 carbon atoms, optionally interrupted by heteroatoms selected from oxygen, sulfur and nitrogen, cycloalkyl, preferably C₃₋₇ cycloalkyl, cycloalkenyl, preferably C₃₋₇ cycloalkenyl, aryl or heteroaryl;

X is C-OH or C=O;

R2 is hydrogen, hydroxy, methyl, ethyl, methoxy or OCOR₄, where R₄ is a straight or branched chain saturated or unsaturated alkyl group, preferably C₁₋₁₀ alkyl, especially C₁₋₆ alkyl, or a cycloalkyl, preferably C₃₋₈ cycloalkyl, or aryl group;

R3 is a straight or branched chain saturated or unsaturated alkyl group, preferably having 3-8 carbon atoms, especially 3-5 carbon atoms, optionally interrupted by one or more heteroatoms selected from oxygen, sulfur and nitrogen, each carbon atom optionally being substituted with a substituent selected from C₁₋₅ alkyl, hydroxy and carbonyl groups, hydroxy and carbonyl preferentially being attached to carbon 15 of the prostaglandin structure, and said alkyl group optionally containing a cycloalkyl, preferably C₃₋₈ cycloalkyl, aryl or heteroaryl group, which may be mono- or independently multi-substituted with C₁₋₃ alkyl, C₁₋₃ alkoxy, hydroxy, nitro, trifluoromethyl or halogen; or a pharmaceutically acceptable salt or ester thereof.

Aryl is preferably substituted or unsubstituted phenyl.

Exemplary heteroaryl groups are thiophene, furan, thiazole, isothiazole, oxazole, isoxazole, pyridine, pyrazine, pyrimidine, pyridazine.

Aryl, heteroaryl and cycloalkyl may be mono- or independently di- or multi-substituted by C₁₋₃ alkyl, C₁₋₃ alkoxy, hydroxy, nitro, trifluoromethyl or halogen.

Unsaturated alkyl may contain one or more double and/or triple bonds.

Halogen is fluorine, chlorine, bromine or iodine, especially fluorine, chlorine or bromine.

The prostaglandins may be in epimeric mixtures as well as in the form of the individual epimers.

The invention is illustrated by means of the following non-limiting examples:

Identification of prostaglandin receptors. The prostaglandin receptors were identified using the reversed transcriptase polymerase chain reaction (RT-PCR) technique. Specific primers were designed for the FP, EP₁, EP₂, EP₃, and TP receptors. The primers used in the assays are presented in Table II. RT-PCR was performed on mRNA isolated from human iridial melanocytes in culture. The cultured cells were used for preparing mRNA. The RT-PCR mix was analysed on agarose gel and bands of expected size were cloned and sequenced. The deduced sequences were analysed for similarity to each prostaglandin receptor sequence.

Methods. Human iridial melanocytes were isolated and cultured according to Hu et al. (1993) and used in early passages. Cells were grown to confluence and harvested for the mRNA enrichment.

mRNA was isolated using Dynals mRNA Direct System (Dynal A/S, Norway) according to the manufacturer's instructions. 100.000-200.000 human melanocyte cells were used in the enrichment. mRNA is covalently bound to an oligo-dT labeled Dynabead. Using reverse transcriptase the first strand cDNA is synthesized directly on

the Dynabeads with the oligo-dT as a reverse transcriptase primer. The second strand cDNA is synthesized using a known 3' sequence primer from respective prostaglandin receptor, resulting in double stranded cDNA. The same set of Dynabeads was used for each receptor RT-PCR. Receptor specific primers were used for PCR amplifying DNA from Dynabead bound cDNA according to the manufacturer's instructions. For the FP and EP₃ receptor reactions the PCR was performed in 50 µl final volume with 5% DMSO, 200 µM dNTPs and 20 pmoles of each primer. For the other receptors hot start with AmpliWax pellets (Perkin Elmer, USA) was used in a final volume of 75 µl with 5% DMSO, 200 µM dNTPs and 20 pmoles of each primer.

Table II. Prostaglandin receptor specific primers.

FP primers:

Primary primers; CAC AAC CTG CCA GAC GGA AAA C and CCA GTC TTT

GAT GTC TTC TGT G

Secondary primers; CAG TAA TCT TCA TGA CAG TGG G and TTG TAG AAA

CAC CAG GTC CTG G

EP₁ primers:

primary primers; TGT GGC ATG GCC GTG GAG and ACC AAC ACC AGC ATT
GGG C

secondary primers; CTG CAG GGA GGT AGA GCT C and GGC ACG TGG TGC
TTC ATC G

EP₂ primers:

primary primers; CAA CCA TGC CTA TTT CTA CAG C and TCT CGC TCC AAA
CTT GGC TG

secondary primers; CTA CGT GGA CAA GCG ATT GGC and TGG TTG ACG
AAC ACT CGC AC

EP₃ primers:

primary primers; GGG ATC CAA GAT CTG GTT CAG and GCC TTC CCG ATC
ACC ATG CTG

secondary primers; CGC AAG AAG TCG TTC CTG CTG and CAC CAA GTC
CCG GGC CAC TG

TP primers:

primary primers; CTG GTG ACC GGT ACC ATC GTG GTG T and GTA GAT CTA
CTG CAG CCC GGA GCG C

secondary primers; TCG CTA CAC CGT GCA ATA CC and GGC TGG AGG GAC
AGC GAC

The PCR mix from these reactions was analyzed on a 1% LMP agarose gel (BioRad Laboratories, USA). DNA fragments of the expected size were TA-cloned using a TA-cloning kit according to the manufacturer's instructions (Invitrogen Inc., USA), and sequenced on an Applied Biosystem Model 373A DNA sequencing system (Applied Biosystems Inc., USA) according to Applied Biosystems' protocol for their Taq Dye Dioxy Terminator cycle sequencing kit. The generated primary data were processed on a VAX computer using the sequence analysis programs from Genetics Computer Group Inc., Madison, USA (Devereux, J., et al., Nucleic Acids Research 12 (1): 387-395 (1984)).

Results: In human iridial melanocytes based on our RT-PCR we could show an expression of FP, EP₂ and EP₃ receptors. However, we were not able to show the presence of the EP₁ and TP receptors (Table III). As positive controls we amplified the expected EP₁ and TP fragments with the same primers from a human kidney

cDNA library. We enriched poly A mRNA from human iridial melanocytes isolated at two different times and performed the PCR reactions several times with identical result.

Table III. RT-PCR (secondary PCR primers) of human iridial melanocyte mRNA using prostaglandin receptor specific primers (see Table II).

Gene	Correct fragment size (bp)		Sequence analysis
	<u>Observed</u>	<u>Expected</u>	
FP	+	489	Identity with FP
EP ₁	-	397	-
EP ₂	+	501	Identity with EP ₂
EP ₃	+	372	Identity with EP ₃
TP	-	484	-

SYNTHESIS OF PROSTAGLANDIN DERIVATIVES

The structures of the end compounds prepared in the Examples are shown in Scheme 1 provided at the end of the description.

Example 1: PGF_{2 β} (compound 1)

The title compound was purchased from Cayman Chemicals Company, Ann Arbor Michigan, USA.

Example 2: PGF_{2 β} isopropyl ester (compound 2)

DBU (163.5 mg, 1.01 mmol) was added to a stirred solution of PGF_{2 β} (Cayman Chemicals) (60 mg, 0.169 mmol) in acetone (20 ml) at 0 °C. The mixture was allowed

to warm to room temperature, when isopropyl iodide (222.6 mg, 1.34 mmol) was added dropwise. After 48 h (TLC monitoring), the mixture was diluted with ethyl acetate (40 ml), washed with brine (30 ml), citric acid 3% (2x40 ml) and sodium hydrogen carbonate 5% (2x30 ml) and dried over anhydrous sodium sulfate. The solvent was removed in vacuo and the residue was chromatographed on silica gel using ethyl acetate : acetone 3:1 as eluent. This afforded a colorless oil, yield 46 mg (68%). ¹H NMR (CDCl₃) d 1.3 (d, 6H), 1.6-1.7 (dm, 4H), 2.0-2.2 (dm, 6H), 2.3 (t, 2H), 4.0-4.1 (m, 3H), 5.0 (sept, 1H), 5.5 (m, 2H), 5.6 (m, 2H). ¹³C NMR (CDCl₃) d 135.9, 132.2, 130.5, 128.0, 75.3, 74.8, 72.85, 67.6, 56.23, 52.25, 51.59, 42.32, 37.35, 33.44, 31.74, 29.14, 26.66, 24.79, 22.6, 21.8, 14.03.

Example 3: 17-Phenyl-18,19,20-trinor-PGE₂ (compound 3)

The title compound was purchased from Cayman Chemicals Company, Ann Arbor Michigan, USA.

Example 4: 17-Phenyl-18,19,20-trinor-PGE₂ isopropyl ester (compound 4)

DBU (43.5 mg, 0.29 mmol) in acetonitrile (1 ml) was added dropwise to a stirred solution of compound 3 (22.1 mg, 0.057 mmol) in acetonitrile (3 ml) at 0 °C. The mixture was allowed to warm to room temperature whereupon isopropyl iodide (78.0 mg, 0.46 mmol) in acetonitrile (2 ml) was added dropwise. After being stirred for 12 h (TLC monitoring), the reaction mixture was quenched with water (8 ml), the mixture was extracted with ethyl acetate (2x50ml), and the extract was washed with brine (10 ml), citric acid 3% (10 ml), and finally sodium hydrogen carbonate 5% (10 ml). After drying with anhydrous sodium sulfate, the solvent was removed in vacuo and the residual oil was chromatographed on silica gel using ethyl acetate as eluent. This afforded 230 mg of the product (69%) of the title compound as a colorless oil: R_f = 0.516 (ethyl acetate : acetone : HOAc 1:1:0.02); ¹H NMR (CDCl₃) d 0.89 (m, 3H), 1.3 (d, 6H), 2.6-2.8 (m, 2H), 4.1 (m, 2H), 5.0 (m, 1H), 5.3-5.7 (dm, 4H), 7.2 (m, 5H). ¹³C NMR (CDCl₃) d 10.9, 13.9, 21.8, 22.9, 23.8, 24.49, 24.8, 25.17, 25.6, 26.68, 28.93, 30.45, 31.77, 33.90, 34.01, 34.07, 38.8, 46.22, 53.3, 54.48, 66.83, 67.62, 68.18, 71.77, 72.21, 76.35, 77.00, 77.2,

77.64, 125.93, 126.46, 128.39, 128.44, 128.79, 130.63, 130.81, 131.04, 137.79, 213.88.

Example 5: 15RS-16,16-trimethylene-PGE₂ (compound 5)

To a stirred solution of 15RS-16,16-trimethylene-PGE₂ methyl ester (52 mg, 0.13 mmol) in acetone (0.4 ml) and phosphate buffer pH 7 (4 ml) was added lipase VII (40 mg). The mixture was stirred at room temperature for 24 h (TLC monitoring). The mixture was quenched with ethanol (3 ml) and extracted with ethyl acetate (2x10 ml). The organic layer was washed with brine, dried (sodium sulfate), and concentrated in vacuo furnishing 46 mg of the product as an oil.

Example 6: 15RS-16,16-trimethylene-PGE₂ methyl ester (compound 6)

The synthesis of 15RS-16,16-trimethylene prostaglandin E₂ (Skotnicki, S. et al. 1977) is schematically shown in Scheme 2. Bold figures in the following refer to respective structures in Scheme 2.

Ethyl 2,2-trimethylenehexanoate (9)

To a stirred solution of N-isopropylcyclohexylamine (56.2 g, 398 mmol) in THF (400 ml) at -78 °C was added rapidly n-BuLi (159 ml, 398 mmol of 2.5 M in hexane). To the resulting solution was added dropwise ethyl cyclobutanecarboxylate (**8**) (50 g, 390 mmol) and stirred for 30 min, then warmed to 0 °C and dropped into a solution of n-butyl iodide (159 ml, 398 mmol of 2.5 mol in hexane) in DMSO (200 ml). The reaction mixture was stirred for 1 h at room temperature (TLC monitoring). The salt was removed by filtration and the filtrate was concentrated in vacuum. The residue was dissolved in hexane and washed with HCl 2%, brine and water, then dried over sodium sulfate, and evaporated in vacuo. The residual oil was distilled (49-56 °C, 1 mmHg) to give 26.5 g (37%) of the product.

¹H NMR (CDCl₃) δ 0.9 (t, 3H), 1.2 (t, 3H), 1.8-2.0 (dm, 5H), 2.2-2.5 (m, 3H), 4.2 (m, 2H).

2,2-Trimethylenehexan-1-ol (10)

To a stirred solution of ethyl 2,2-trimethylenehexanoate (**9**) (26.5 g, 144 mmol) in dry toluene (100 ml) was added dropwise DIBAL-H (206 ml, 289 mmol of 1.4 mol in toluene) at 0 °C. The resulting solution was stirred at room temperature for 3 h (TLC monitoring), and then poured into iced HCl 5%. The organic layer was separated and washed with HCl 5%, brine, dried, filtered and concentrated to give 30 g of the product as an oil. ^1H NMR (CDCl_3) δ 0.9 (t, 3H), 1.8-2.0 (dm, 5H), 2.5 (m, 1H), 3.0 (m, 1H), 3.6 (m, 2H).

2,2-Trimethylenehexaldehyde (**11**)

To a solution of 2,2-trimethylenehexan-1-ol (**10**) (30 g, 210 mmol), in DME (400 ml), was added dicyclohexanecarbodiimide (DCC) (130 g, 630 mmol), DMSO (120 ml) and orthophosphoric acid (10.3 g). The mixture was stirred at room temperature for 3 h (TLC monitoring), and filtered. The filtrate was diluted with dichloromethane (300 ml), and washed with water. The organic layer was separated. The residue was removed by filtration. The filtrate was washed with brine (100 ml), dried and concentrated in vacuum. The residue was purified by column chromatography on silica gel using hexane as eluent to give the title product (17.3 g) as an oil.

^1H NMR (CDCl_3) δ 0.9 (t, 3H), 1.2 (t, 3H), 1.8-2.0 (dm, 5H), 8.8 (s, 1H).

4,4-Trimethylene-1-octyn-3-ol (**12**)

To a solution of lithium acetylide-ethylenediamine complex (12.2 g, 132 mmol) in DMSO (10 ml) was added a solution of 2,2-trimethylenehexaldehyde (**11**) (17 g, 120 mmol) in DMSO (20 ml) at 0 °C under N_2 . The mixture was stirred at room temperature for 24 h (TLC monitoring) and then poured into an ice-cold HCl 2% (50 ml) and ether (50 ml). The organic layer was separated and the aqueous layer was extracted with ether (50 ml), the combined organic phases were washed with brine, dried, filtered and concentrated in vacuo. The residue was chromatographed on silica gel using hexane : ethyl acetate 5:1 as eluent, which gave **12** (7.6 g, 38%) as an oil.

E-Tributyltin-4,4-trimethylene-1-octene-3-ol (**13**)

A mixture of 4,4-trimethylene-1-octyn-3-ol (**12**) (5.0 g, 30 mmol), tributyltin hydride (14.6 ml, 54.2 mmol), and AIBN (30 mg) was stirred at 130 °C for 24 h (TLC monitoring). The residue was chromatographed on silicagel using hexane and hexane:ether 9:1, respectively, as eluent, to give the title compound (**13**) (12.54 g, 91.4%) as an oil.

E-Tributyltin-4,4-trimethylene-3-trimethylsilyloxy-1-octene (**14**)

To the mixture of E-tributyltin-4,4-trimethylene-1-octene-3-ol (**13**) (7 g, 15.3 mmol) in DMF (100 ml) was added imidazole (2.1 g, 30.6 mmol) and trimethylsilyl chloride (2.5 g, 23.0 mmol). The reaction mixture was stirred at room temperature for 1 h (TLC monitoring). The mixture was partitioned between water (200 ml) and ether (200 ml). The organic phase was dried and evaporated in vacuo. The residue was chromatographed on silica gel using hexane as eluent to give **14** (5.53 g).

11,15-bis Trimethylsilyloxy-16,16-trimethylene-5,6-didehydro-PGE₂ methyl ester (**17**)

A dry 100-ml three-necked flask was charged with copper(I)cyanide (928 mg, 10.4 mmol) and a magnetic bar. The flask was capped with a rubber septum and heated under vacuum to remove any trace of water, and cooled to 0 °C under N₂. Dry THF was added and followed by methyl lithium (14 ml, 22.4 mmol of 1.6 mol in diethyl ether), via a syringe. The mixture was stirred at 0 °C for 15 min. during which the suspension became clear and homogeneous. A solution of E-tributyltin-4,4-trimethylene-3-trimethylsilyloxy-1-octene (**14**) (5.9 g, 11.2 mmol) in THF (10 ml) was added, via a syringe, at 0 °C and stirred at room temperature for 30 min. To the resulting solution a solution of 4-(t-butyldimethylsilyloxy)-cyclopentenone (**15**) (1.7 g, 8 mmol) in THF (6 ml), trimethylsilyl chloride (4.35 g, 40 mmol) and triethylamine (8.1 g, 80 mmol) was added, at -70 °C, successively and stirred at -70 °C for another 15 min, then for 15 min at 0 °C. The mixture was partitioned between hexane (600 ml) and water (300 ml). The organic layer was separated, dried over sodium sulfate, filtered and concentrated in vacuo to give the crude silyl enol ether as a clear oil. To the stirred solution of the silyl enol ether in THF (50 ml), under N₂, at -30 °C methyl lithium (7.7 ml, 12.3 mmol of 1.6 mol in diethyl ether) was added and stirred for 30 min followed by addition of a freshly prepared methyl-1-triflate-2-hexynoate (**16**)

(Erhardt, P.W., et al. 1987; Caldwell A. G., et al. 1979) and stirred at -40 °C for 5 min. The resulting solution was quenched with saturated aqueous ammonium chloride solution (30 ml) and extracted with ether (3x 100 ml), dried on sodium sulfate, filtered and concentrated in vacuo. The residue was chromatographed on silica gel using hexane : ethyl acetate (1:1) as eluent, to give a clear oil of a mixture of 15RS isomers (2.71 g, 57.3%) R_f = 0.36 (SiO₂, ether : hexane 1:1). ¹H NMR (CDCl₃) δ 0.2 (dm, 12H), 0.8-0.9 (ms, 18H), 1.8 (m, 2H), 2.3 (m, 4H), 3.7 (s, 3H), 3.9-4.1 (dm, 2H), 5.5-5.6 (2H). The ¹H NMR was also performed on the desilylated analogue, 16,16-trimethylene-5,6-didehydro-PGE₂ methyl ester. ¹H NMR (CDCl₃) δ 0.9 (t, 3H), 1.2-1.3 (m, 3H), 1.9-2.1 (m, 4H), 3.7 (s, 3H), 4.1 (m, 2H), 5.6-5.9 (dm, 2H).

11,15-bis Trimethylsilyloxy-16,16-trimethylene-PGE₂ methyl ester

To a stirred solution of 11,15-bis trimethylsilyloxy-16,16-trimethylene-5,6-didehydro-PGE₂ methyl ester (**17**) (500 mg, 0.8 mmol), in benzene : cyclohexane 1 : 1 (50 ml) was added Pd-BaSO₄ (250 mg) and quinoline (250 mg) and stirred at -40 °C under H₂ atmosphere for 5 h (TLC monitoring). The reaction mixture was diluted with ether and filtered through celite, and concentrated in vacuum. The residue was chromatographed on silica gel using hexane : ethyl acetate 9:1 to give 442 mg of the corresponding product.

16,16-Trimethylene-PGE₂ methyl ester (**6**)

To the solution of 11,15-bis trimethylsilyloxy-16,16-trimethylene-PGE₂ methyl ester (374 mg, 0.589 mmol) in THF (18 ml) was added HF 40% (3.5 ml) in THF (1 ml) at 0 °C. The reaction mixture was stirred for 5 h (TLC monitoring) and then poured into a mixture of sodium hydrogen carbonate 5% (30 ml) and ethyl acetate (50 ml). The organic layer was separated and the aqueous layer was washed with ethyl acetate (2x30 ml). The organic layers were pooled and dried, on sodium sulfate, and concentrated in vacuo. The residue was chromatographed on silica gel using hexane : ethyl acetate 1:1, and ethyl acetate successively to give **6** (75 mg, 31%), as an oil.

¹H NMR (CDCl₃) δ 0.9 (t, 3H), 1.3 (t, 6H), 2.0-2.6 (mm, 9H), (dm, 5H), 3.6 (s, 3H), 4.1 (m, 2H), 5.4 (m, 2H), 5.6-5.8 (dm, 2H); ¹³C NMR (CDCl₃) δ 14.222, 14.9, 23.7,

24.7, 25.2, 26.2, 26.5, 26.6, 26.8, 29.7, 33.4, 36.5, 44.9, 46.0, 51.6, 54.0, 54.6, 71.9, 76.7, 77.06, 77.1, 77.38, 126.5, 126.9, 127.7, 130.9, 132.5, 132.9, 133.36, 133.46, 174.15, 214.32.

Example 7: Synthesis of 13,14-dihydro-17-(3-fluorophenyl)-18,19,20-trinor PGE₂ isopropyl ester (compound 7)

The synthesis of the title compound is schematically shown in Scheme 3. Bold figures refer to respective structures in Scheme 3.

Dimethyl-(2-oxo-4-(3-fluorophenylbutyl) phosphonate

To a stirred suspension of sodium hydride (4.17 g, 138 mmol) previously washed with n-pentane, in dry THF (250 ml) at room temperature was added dropwise a solution of dimethyl-2-oxo-propylphosphonate (23.12 g, 132.3 mmol) in THF (110 ml). The reaction mixture was stirred for 2 h, then cooled in an ice bath and treated with a solution of n-BuLi (10.2 g, 158.7 mmol) in hexane, causing a dark brown solution to be formed. Stirring was continued for 2 h at 0 °C, followed by dropwise addition of 3-fluorobenzyl bromide (25 g, 132.3 mmol) in THF (50 ml). The reaction mixture was gradually warmed to room temperature and after 3 h (TLC monitoring), it was quenched with 10% HCl (20 ml). The mixture was poured into ice-water (200 ml), extracted with CHCl₃ (2x150 ml), the organic layers were collected, washed with brine (150 ml), chromatographed on silica gel using CH₂Cl₂ and EtOAc successively as eluent, furnishing 19.5 g of a slightly yellow oil. *R*_f = 0.37 (silica gel, EtOAc:acetone 1:1)

(1S,5R,6R,7R)-6-Formyl-7-(4-phenyl benzyloxy)-2-oxabicyclo[3.3.0]octane-3-one

19

To a solution of the alcohol **18** (19.0 g, 53.9 mmol) in DME (100 ml), cooled to 18 °C, was added dicyclohexylcarbodiimide (DCC) (33.3, 161.8 mmol), DMSO (38.2 ml) and phosphoric acid (1.43 ml, 21.28 mmol). The temperature of the reaction mixture was kept below 25 °C for 30 min. The reaction mixture was stirred at room temperature for additional 2 hours (TLC monitoring), and the precipitate was removed

by filtration and washed with ether (2x50 ml). The combined organic layer was washed with water (50 ml) and brine (2x50 ml), the aqueous solution was extracted with ether (100 ml), the organic layers were collected and dried over sodium sulfate, filtered, and used directly for the next step. TLC R_f = 0.37 (silica gel, EtOAc:toluene 2:1).

(1S,5R,6R,7R)-6-{3-Oxo-5-(3-fluorophenyl)-1-E-pentenyl}-7-(4-phenyl benzoyloxy)-2-oxabicyclo[3.3.0]octane-3-one (20)

To a stirred suspension of NaH (1.9 g, 65.1 mmol), prewashed with n-pentane, in DME (130 ml) under nitrogen, was added dropwise dimethyl-2-oxo-4-(3-fluorophenyl) butylphosphonate (Wadsworth, Jr., W. S., et al. 1961) (19.3 g, 70.5 mmol), in DME (100 ml) and stirred vigorously for 1 h at room temperature. The mixture was then cooled to -10 °C and a solution of the crude aldehyde **19** was added dropwise. After 30 min. at 0 °C and 2 h at room temperature (TLC monitoring), the reaction mixture was neutralized with acetic acid, the solvent was removed in vacuo and the residue was dissolved in EtOAc (200 ml), and washed with water (50 ml) and brine (50 ml). The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated in vacuo. The residue was stirred with ether (100 ml), the resulting white precipitate was filtered and washed with cold ether, giving a white crystalline substance yield (17 g, 58.5%) R_f = 0.56 (silica gel, ethyl acetate : toluene 2:1)

(1S,5R,6R,7R)-6-(3S-3-Hydroxy-5-(3-fluorophenyl)-1-pentenyl)-7-(4-phenyl benzoyloxy)-2-oxabicyclo[3.3.0]octane-3-one (21)

To a stirred solution of the enone **20** (17.1 g, 34.3 mmol) in THF (20 ml) and cerium chloride ($\text{CeCl}_3 \cdot 7 \text{ H}_2\text{O}$) (3.8g, 10.3 mmol) in THF:ether 1:2 (60 ml) cooled to -20 °C under nitrogen was added sodium borohydride (0.8 g, 20.57 mmol) in small portions. The reaction mixture was stirred for 2 h (TLC monitoring). The temperature was raised to ±0 °C, then quenched by adding water (20 ml) and an aqueous solution of 10% HCl to pH 4 and extracted with EtOAc (50 ml). The organic layer was separated and washed with brine, dried on anhydrous sodium sulfate, concentrated in vacuo and

chromatographed twice on silica gel using toluene:EtOAc 2:1 and 1:1 successively as eluent, furnishing **4** (5 g) as a white crystalline product $R_f = 0.32$ (silica gel, EtOAc:toluene 2:1).

(1S,5R,6R,7R)-6-{3R-3-Hydroxy-5-(3-fluorophenyl)-1-pentyl}-7-(4-phenylbenzoyloxy)-2-oxabicyclo[3.3.0]octane-3-one (22)

To a suspension of 10% Pd/C (0.1 g) in sodium nitrite (3.6 ml, 1.8 mmol) and ethanol (15 ml) was added a solution of **21** (3 g, 6.0 mmol) in ethanol (6.0 ml). The mixture was stirred under hydrogen atmosphere for 6 h (TLC monitoring), and quenched with 1M solution of HCl. The catalyst was removed by filtration through a celite pad, washed with ethanol abs. (15 ml). The solvent was removed in vacuo. The resulting oil was dissolved in EtOAc (100 ml), and washed with brine 15% (30 ml). The water phase was washed with EtOAc (40 ml). The combined organic extracts were dried over sodium sulfate and filtered. The solvent was removed in vacuo. The residue was chromatographed on silica gel using EtOAc as eluent, which gave **5** (2.94 g), $R_f = 0.25$ (silica gel, EtOAc).

(1S,5R,6R,7R)-6-{3R-3-Hydroxy-5-(3-fluorophenyl)-1-pentyl}-7-R-hydroxy-2-oxabicyclo[3.3.0]octane-3-one (23)

To a solution of the lactone **22** (2.8 g, 5.65 mmol) in methanol (15 ml) was added potassium carbonate (0.47 g, 3.3 mmol) and the mixture was stirred at ambient temperature for 6 h (TLC monitoring). The mixture was neutralised with 10% aqueous solution of HCl and extracted with EtOAc (2x30 ml). The organic phase was dried on anhydrous sodium sulfate and evaporated to dryness. The crude product was chromatographed on silica gel using EtOAc:acetone 1:1 as eluent. The title compound **23** was obtained as a white crystalline product; yield 1.6 g, $R_f = 0.17$ (silica gel, EtOAc); ^1H NMR (CDCl_3) δ 1.2-1.4 (m, 1H), 1.54 (m, 3H), 1.8 (m, 3H), 2.1 (m, 1H), 2.2 (m, 1H), 2.3 (m, 1H), 2.6 (m, 2H), 2.67 (m, 1H), 2.8 (m, 2H), 3.60 (m, $\text{CH}_2\text{CHOHCH}_2$), 4.0 (m, CHOH) 4.92 (m, CHOC=O), 6.8-7.0 (m, 3H), 7.28 (m, 1H).

(1S,5R,6R,7R)-6-{3R-3-t-butyl dimethylsilyloxy-5-(3-fluorophenyl)-1-pentyl}-7-R-t-butyl dimethylsilyloxy-2-oxabicyclo[3.3.0]octane-3-one (24)

t-Butyldimethylsilyl chloride (2.3 g, 14.9 mmol) was added in one portion to a solution of the diol 23, triethyl amine (2.1 ml, 14.8 mmol) and 4-dimethylamino pyridine (0.06 g, 0.1 mmol) in dichloromethane (20 ml) with vigorous stirring at room temperature for 24 h, and the reaction mixture was concentrated in vacuo. The crude product was dissolved in ethyl acetate (50 ml), washed with water (20 ml) and 5% aqueous solution of sodium hydrogen carbonate (20 ml). The organic phase was dried on sodium sulfate, filtered and concentrated in vacuo. The residue was chromatographed on silica gel using dichloromethane as eluent to give 3 g of the product as oil. $R_f = 0.68$ (silica gel, ether)

(1S,5R,6R,7R)-6-{3R-3-t-butyl dimethylsilyloxy-5-(3-fluorophenyl)-1-pentyl}-7-R-t-butyl dimethylsilyloxy-2-oxabicyclo[3.3.0]octane-3-ol (25)

A solution of diisobutylaluminium hydride (DIBAL) (1.1 g, 7.43 mmol) in dry toluene (5.3 m) was added dropwise to a stirred solution of the lactone 24 (2.7 g, 4.95 mmol) in dry THF (30 ml) at -72/-80 °C. After 1 h (TLC monitoring), the reaction mixture was quenched with methanol (5 ml) and was warmed to room temperature, and added water (50 ml), 10% aqueous solution of HCl (50 ml), extracted with EtOAc (2x50 ml). The organic layer was dried with sodium sulfate, filtered, the solvent was removed in vacuo, and the residue was chromatographed on silica gel using EtOAc and EtOAc:acetone 1:1, respectively, as eluent, to give a yellow oil product (2.7 g), $R_f = 0.85$ (silica gel, ethyl acetate 1:1).

13,14-Dihydro-11,15-di-t-butyldimethyl silyloxy-17-(3-fluorophenyl)-18,19,20-trinor-PGF_{2α} (26)

To a stirred suspension of 4-carboxybutyl triphenyl phosphonium bromide (8.78 g, 19.82 mmol) in THF (50 ml) under nitrogen at 0-5 °C was added potassium t-butoxide (3.89 g, 34.6 mmol), and the mixture stirred for 30 min. at room temperature. To the resultant red orange solution of ylide at -15/-10 °C was added the lactol 25 (2.7 g, 4.95 mmol) in THF (10 ml), and the mixture was stirred for 3-4 h (TLC monitoring). The reaction mixture was diluted with water (30 ml) and washed with ether (4x40 ml). The water layer was acidified with 5% aqueous solution of citric acid to pH 4 and extracted with EtOAc (2x50 ml). The organic phase was washed

with brine (30 ml), dried on sodium sulfate, and filtered. The solvent was removed in vacuo, and the slurry **26** was used directly without isolation for the next step.

13,14-Dihydro-11,15-di-t-butyl dimethyl silyloxy 17-(3-fluorophenyl)-18,19,20-trinor PGF_{2α} isopropyl ester (27)

DBU (5.28 g, 34.7 mmol) was added dropwise to a stirred solution of the crude product **26** (3.16 g, 4.96 mmol) in acetone (20 ml) at 0 °C. The mixture was allowed to warm to room temperature, and isopropyl iodide (5.05 g, 29.7 mmol) was added dropwise. After 4 h (TLC monitoring), the mixture was diluted with EtOAc (100 ml), washed with brine (30 ml), citric acid 3% (2x25 ml) and sodium hydrogen carbonate 5% (2x25 ml) and dried over anhydrous sodium sulfate. The solvent was removed in vacuo and the residue was chromatographed on silica gel using ether:petroleum ether 1:2 as eluent. This afforded a colourless oil, yield 1.7 g, $R_f = 0.43$ (silica gel, ether : petroleum ether 1:2). ¹H NMR (CDCl₃) δ 0.1 (m, 9H), 0.9 (m, 16H), 1.2 (m, 9H), 1.6-1.8 (mm, 10H), 2.12 (m, 2H), 2.22-2.33 (m, 2H), 2.6-2.9 (dm, 2H), 3.65 (m, CH₂CHOHCH₂), 3.94 (m, CH₂CHOH), 4.16 (m, CH₂CHOH), 5.0 (sept. 1H), 5.38 (m, db), 5.47 (m, db), 6.8-7.0 (dm, Ar, 3H), 7.2 (m, Ar, 1H).

13,14-Dihydro-11,15-di-t-butyl dimethyl silyloxy 17-(3-fluorophenyl)-18,19,20-trinor PGE₂ isopropyl ester (28)

Pyridinium dichlorochromate (2.43 g, 11.25 mmol) on aluminum oxide (20 g) was added in small portions to a solution of **27** (1.7 g, 2.5 mmol) in dichloromethane (30 ml) and the mixture was stirred at room temperature (TLC monitoring), filtered, and the precipitate was washed with ether: ethyl acetate 2:1. The solvent was removed in vacuo. The residue was diluted with ether (100 ml) and washed with water (30 ml), 5% aqueous solution of NaHCO₃ (3x20 ml), the organic phase was separated and dried over sodium sulfate, and evaporated in vacuo to give **28** (1.3 g), as an oil. $R_f = 0.72$ (silica gel, ethyl acetate).

13,14-Dihydro-17-(3-fluorophenyl)-18,19,20-trinor PGE₂ isopropyl ester (7)

Hydrogen fluoride 15% (12 ml) was added to a solution of 28 (314 mg) in acetonitrile. The mixture was stirred at room temperature for 4 h (TLC monitoring). The reaction mixture was diluted with ethyl acetate (100 ml) and washed with water (3x20 ml), dried and evaporated in vacuo. The residue was chromatographed on silica gel using ethyl acetate as eluent, which gave 7 (64 mg) as an oil, R_f = 0.43 (silica gel, ethyl acetate). ^1H NMR (CDCl_3) δ 1.2 (d, 6H), 1.6-1.8 (m, 6H), 1.8 (m, 2H), 2.12 (m, 2H), 2.2-2.3 (m, 2H), 2.6-2.8 (dm, 2H), 3.6 (m, $\text{CH}_2\text{CHOHCH}_2$), 4.16 (m, CH_2CHOH), 5.0 (sept. 1H), 5.38 (m, db), 5.47 (m, db), 6.8-7.0 (dm, Ar, 3H), 7.2 (m, Ar, 1H).

Pharmacology

Intraocular pressure reducing effect of he test compounds in cats and monkeys.

The compounds were tested for intraocular pressure reducing effect in animal models. The intraocular pressure was measured with a calibrated pneumotonometer. European domestic cats and cynomolgus monkeys were used as experimental animals. The cornea was anaesthetized with oxibuprocaïn before the measurement. The reductions in intraocular pressure after topical treatment with the PGF_{2 β} isopropyl ester (2), 17-phenyl-18,19,20-trinor-PGE₂-isopropyl ester (4), 15RS-16,16-trimethylene-methyl ester (6) and 13,14-dihydro-17-(3-fluorophenyl)-18,19,20-trinor-PGE₂-isopropyl ester (7) are demonstrated in Tables IV and V.

Table IV. Intraocular pressure reducing effect of 1-10 µg of the test compounds, with effect on the EP₁ prostanoid receptor, in cats. The control eye received the vehicle only. (n= 5-6; Mean±SEM).

Prostaglandin/ Eye	Baseline pressure (mmHg)	Pressure 3 h after treatment (mmHg)
<u>2</u>		
Experimental eye	24.2 ± 2.3	15.1 ± 2.8 *
Control eye	24.5 ± 2.7	22.5 ± 3.4
<u>4</u>		
Experimental eye	22.0 ± 1.7	14.2 ± 1.7 *
Control eye	21.5 ± 1.7	18.7 ± 1.9
<u>6</u>		
Experimental eye	19.2 ± 1.7	9.5 ± 0.5 *
Control eye	19.3 ± 1.7	17.0 ± 1.3
<u>7</u>		
Experimental eye	20.4 ± -2.0	14.2 ± 0.9 *
Control eye	20.6 ± -1.8	18.4 ± 1.5

* p<0.01 (matched pair t-test between eyes)

Table V. Intraocular pressure reducing effect of the test compounds, with effect on the EP₁ receptor, in monkeys. The dose of PGF_{2β}-isopropyl ester was 30 µg, while that of 17-phenyl-18,19,20-trinor-PGE₂-isopropyl ester, and 15RS-16,16-trimethylene-PGE₂-isopropyl ester was 3 µg. The control eye received the vehicle only (n=6; Mean±SEM).

Prostaglandin/ Eye	Baseline pressure (mmHg)	Pressure 4 h after treatment (mmHg)
<u>2</u>		
Experimental eye	17.8 ± 1.4	14.1 ± 1.8 *
Control eye	16.9 ± 1.2	17.5 ± 2.0
<u>4</u>		
Experimental eye	14.1 ± 1.1	9.9 ± 0.9 *
Control eye	13.9 ± 1.0	11.5 ± 0.8
<u>6</u>		
Experimental eye	20.9 ± 1.6	15.3 ± 2.4 *
Control eye	21.3 ± 1.5	19.0 ± 1.5

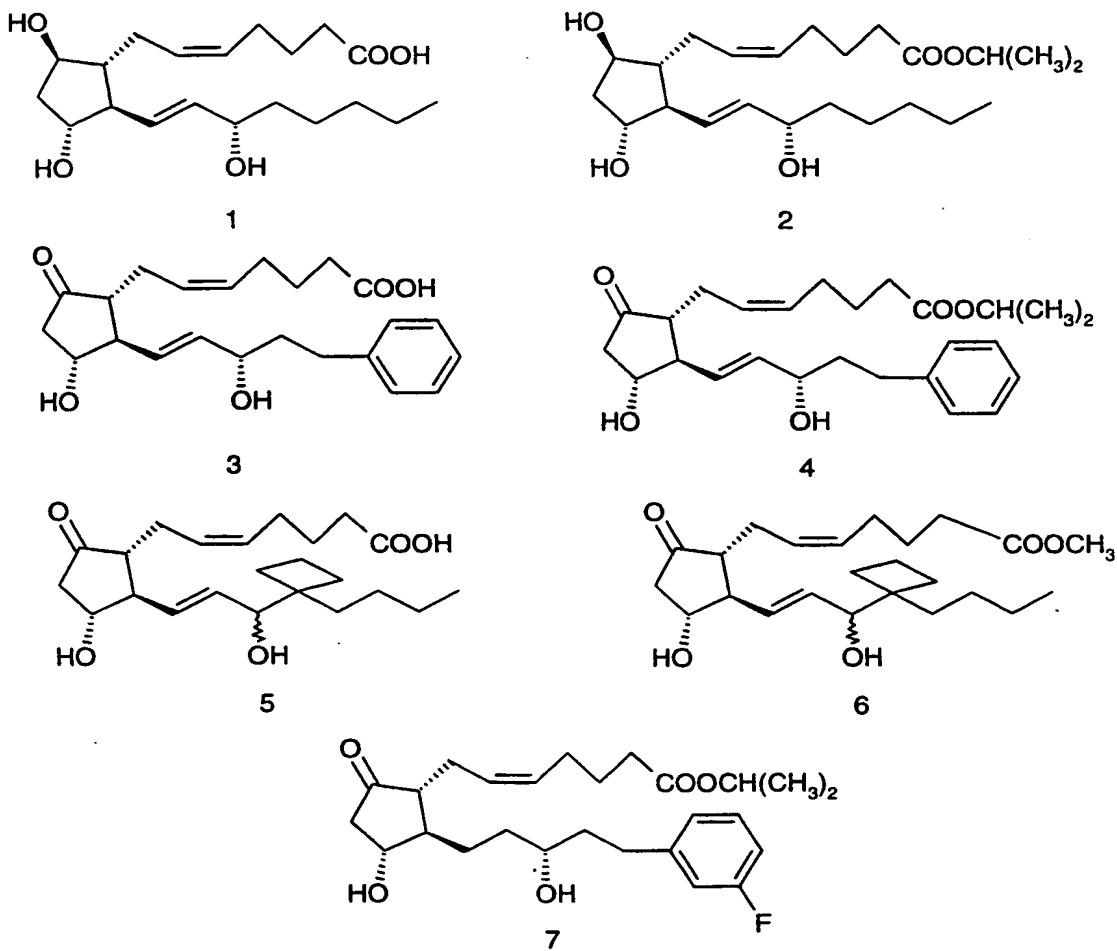
* p<0.05 (match paired t-test between the eyes)

It can be seen that both in cats and monkeys all the prostaglandin analogues with preference for the EP₁ receptor significantly reduced the intraocular pressure.

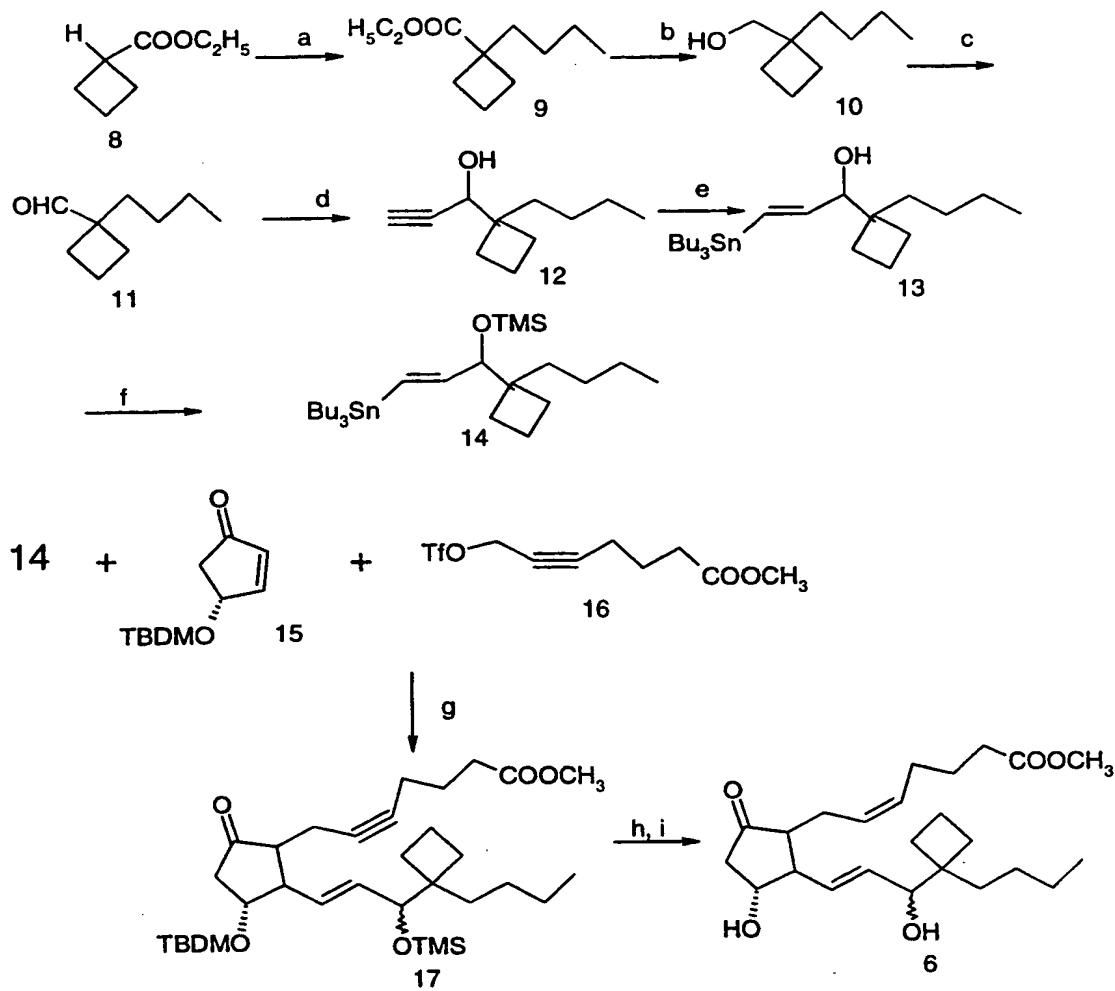
Accordingly, the present invention discloses that compounds with selective stimulatory effect on EP₁ receptors reduce the intraocular pressure, and that such compounds cannot have any melanogenic effect, or at least have significantly reduced effect in the eye since the pigment producing cells, the melanocytes, lack the EP₁

receptor in man. Thus, the common side-effect of increased iridial pigmentation can be avoided during chronic therapy with prostaglandins selective for EP₁ receptors.

Scheme 1



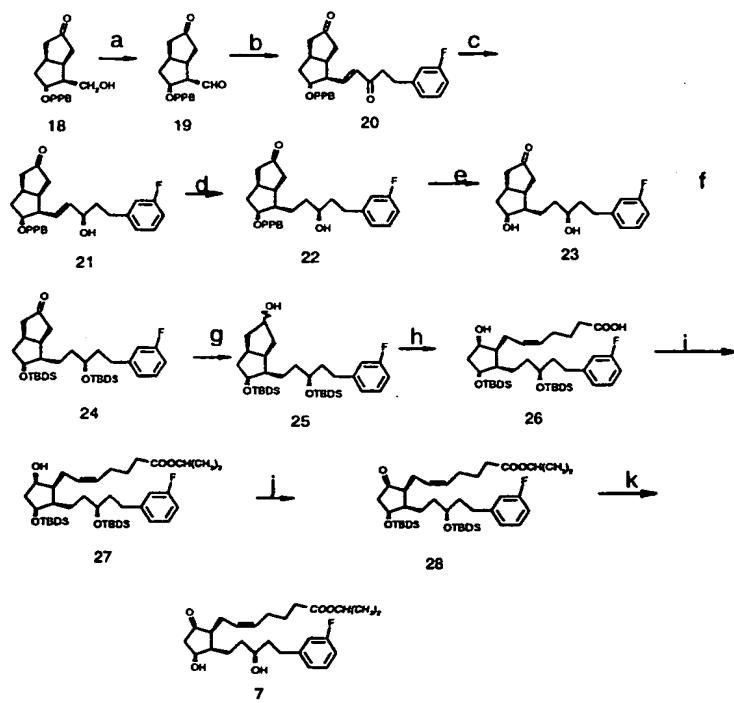
Scheme 2



Reagents

- a. N-isopropylcyclohexyl amine / THF, n-BuLi, ethylcyclobutanecarboxylate/ DMSO
- b. DIBAL-H, / toluene
- c. DCC/ DME, DMSO, H₃PO₄,
- d. Lithium-acetylide-ethylene diamine, DMSO
- e. tributyltin hydride, AIBN
- f. Trimethylsilyl chloride (TMSCl), imidazole/ DMF
- g. $\text{Li}_2\text{CuCN}(\text{CH}_3)_2$, TMSCl, triethylamine, 4-t-butyl-dimethylsilyloxy-2-cyclopentenone, 1-tributyltin-4,4,-trimethylene-3-trimethylsilyloxy-1-octene, methyl-2-yn-8-octanoate
- h. Pd-BaSO₄, quinoline,
- i. HF/THF

Scheme 3



Reagents

- a.DCC, DMSO, H₂SO₄, DME, H₃PO₄
- b.NaH, dimethyl-2-oxo-4-(3-fluorophenyl)-butylphosphonate
- c. NaBH₄, CeCl₃.7H₂O/THF
- d.Pd/C, NaNO₂/ THF
- e.K₂CO₃/Methanol
- f.TBDMS, TEA, 4-dimethylamino pyridine/dichloromethane
- g.DIBAL-H/ THF
- h.4-carboxybutyl triphenyl phosphonium bromide
potassium t-butoxide, THF
- i.DBU, isopropyl iodide/ acetone
- j.pyridinium chlorochromate, aluminium oxide/ dichloromethane
- k.HF/acetonitrile

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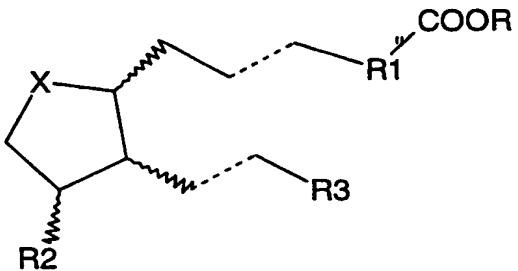
Stjernschantz, J. and Alm, A. (1996). Latanoprost as a new horizon in the medical management of glaucoma. *Current Opinion in Ophthalmology* 7; 2: 11-17.

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CLAIMS

1. A composition for the treatment of glaucoma and ocular hypertension comprising a therapeutically active and physiologically acceptable amount of a prostaglandin analogue which is a selective agonist for EP₁ prostanoid receptors, or a pharmaceutically acceptable salt or ester thereof.
2. The composition according to claim 1, wherein the prostaglandin analogue is derived from PGF or PGE type prostaglandins.
3. The composition according to claim 1 or 2, wherein the prostaglandin analogue is a compound of the general formula:



wherein:

the wavy bonds represent the α or β configuration, and the dashed bonds represent a single bond, a triple bond or a double bond in the cis or trans configuration;

R is hydrogen, saturated or unsaturated alkyl, preferably C₁₋₁₀ alkyl, cycloalkyl, preferably C₃₋₈ cycloalkyl, aryl, arylalkyl, preferably aryl-C₂₋₅ alkyl, or heteroaryl;

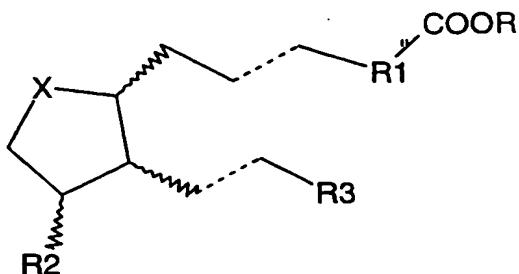
R1 is a saturated or unsaturated alkyl group having 2-5 carbon atoms, optionally interrupted by a heteroatoms selected from oxygen, sulfur and nitrogen, cycloalkyl, preferably C₃₋₇ cycloalkyl, cycloalkenyl, preferably C₃₋₇ cycloalkenyl, aryl or heteroaryl;

X is C-OH or C=O;

R2 is hydrogen, hydroxy, methyl, ethyl, methoxy or OCOR4, where R4 is a straight or branched chain saturated or unsaturated alkyl group, preferably C₁₋₁₀ alkyl, especially C₁₋₆ alkyl, or a cycloalkyl, preferably C₃₋₈ cycloalkyl, or aryl group;

R3 is a straight or branched chain saturated or unsaturated alkyl group, preferably having 3-8 carbon atoms, especially 3-5 carbon atoms, optionally interrupted by one or more heteroatoms selected from oxygen, sulfur and nitrogen, each carbon atom optionally being substituted with a substituent selected from C₁₋₅ alkyl, hydroxy and carbonyl groups, hydroxy and carbonyl preferentially being attached to carbon 15 of the prostaglandin structure, and said alkyl group optionally containing a cycloalkyl, preferably C₃₋₈ cycloalkyl, aryl or heteroaryl group, which may be mono- or independently multi-substituted with C₁₋₃ alkyl, C₁₋₃ alkoxy, hydroxy, nitro, trifluoromethyl or halogen; or a pharmaceutically acceptable salt or ester thereof.

4. The composition according to claim 1, 2 or 3, wherein the prostaglandin analogue is 15(R,S)-16,16-trimethylene-PGE₂ or an alkyl ester thereof.
5. The composition according to claim 1, 2 or 3 wherein the prostaglandin analogue is 13,14-dihydro-17-(3-fluorophenyl)-18,19,20-trinor-PGE₂ or an alkyl ester thereof.
6. A method of treating glaucoma or ocular hypertension in a subject's eye, which method comprises contacting the surface of the eye with an effective intraocular pressure reducing amount of a therapeutically active and physiologically acceptable prostaglandin analogue which is a selective agonist for EP₁ prostanoid receptors, or a pharmaceutically acceptable salt or ester thereof.
7. The method according to claim 6, wherein the prostaglandin analogue is derived from PGF or PGE prostaglandins.
8. The method according to claim 6 or 7, wherein the prostaglandin analogue is a compound of the general formula:



wherein:

the wavy bonds represent the α or β configuration, and the dashed bonds represent a single bond, a triple bond or a double bond in the cis or trans configuration;

R is hydrogen, saturated or unsaturated alkyl, preferably C₁₋₁₀ alkyl, cycloalkyl, preferably C₃₋₈ cycloalkyl, aryl, arylalkyl, preferably aryl-C₂₋₅ alkyl, or heteroaryl;

R1 is a saturated or unsaturated alkyl group having 2-5 carbon atoms, optionally interrupted by a heteroatoms selected from oxygen, sulfur and nitrogen, cycloalkyl, preferably C₃₋₇ cycloalkyl, cycloalkenyl, preferably C₃₋₇ cycloalkenyl, aryl or heteroaryl;

X is C-OH or C=O;

R2 is hydrogen, hydroxy, methyl, ethyl, methoxy or OCOR₄, where R₄ is a straight or branched chain saturated or unsaturated alkyl group, preferably C₁₋₁₀ alkyl, especially C₁₋₆ alkyl, or a cycloalkyl, preferably C₃₋₈ cycloalkyl, or aryl group;

R3 is a straight or branched chain saturated or unsaturated alkyl group, preferably having 3-8 carbon atoms, especially 3-5 carbon atoms, optionally interrupted by one or more heteroatoms selected from oxygen, sulfur and nitrogen, each carbon atom optionally being substituted with a substituent selected from C₁₋₅ alkyl, hydroxy and carbonyl groups, hydroxy and carbonyl preferentially being attached to carbon 15 of the prostaglandin structure, and said alkyl group optionally containing a cycloalkyl, preferably C₃₋₈ cycloalkyl, aryl or heteroaryl group, which may be mono- or independently multi-substituted with C₁₋₃ alkyl, C₁₋₃ alkoxy, hydroxy, nitro, trifluoromethyl or halogen; or a pharmaceutically acceptable salt or ester thereof.

9. The composition according to claim 6, 7 or 8, wherein the prostaglandin analogue is 15(R,S)-16,16-trimethylene-PGE₂ or an alkyl ester thereof.

10. The composition according to claim 6, 7 or 8 wherein the prostaglandin analogue is 13,14-dihydro-17-(3-fluorophenyl)-18,19,20-trinor-PGE₂ or an alkyl ester thereof.
11. The method according to any one of claims 6-10, wherein a therapeutically active and physiologically acceptable composition containing said prostaglandin analogue is administered topically on the eye 1-3 times daily.
12. Use of a prostaglandin analogue which is a selective agonist for EP₁ prostanoid receptors as defined in any one of claims 1 to 4 for the preparation of a medicament for treatment of glaucoma and ocular hypertension.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/01368

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 31/557

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9408585 A1 (ALCON LABORATORIES, INC.), 28 April 1994 (28.04.94) --	1-3,12
X	Journal of Lipid Mediators, Volume 6, 1993, David F. Woodward et al, "Intraocular pressure effects of selective prostanoid receptor agonists involve different receptor subtypes according to radioligand binding studies" page 545 - page 553 --	1-3,12
A	The Journal of Biological Chemistry, Volume 268, No 27, Sept 1993, Akiko Watabe et al, "Cloning and Expression of cDNA for a Mouse EP1 Subtype of Prostaglandin E Receptor", page 20175 - page 20178 --	12

 Further documents are listed in the continuation of Box C. See patent family annex.

- * Special categories of cited documents:
- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
- "&" document member of the same patent family

Date of the actual completion of the international search

12 October 1998

Date of mailing of the international search report

01-11-1998

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/01368

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Natural product reports, Volume 7, No 5, 1990, D. E. Bays et al, "Inhibitors of Gastric Acid Secretion", page 409 - page 445, see page 436 --	12
X	US 4132738 A (HAROLD C. KLUENDER ET AL), 2 January 1979 (02.01.79) -- -----	1-4

INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/01368

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 6-8, 11
because they relate to subject matter not required to be searched by this Authority, namely:
A method for treatment of the human or animal body by therapy, see rule 39.1.
2. Claims Nos.: 12
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The expression "a selective agonist for EP1 prostanoid receptors" in claim 12 is indefinite. According to PCT Article 6, the claims shall be clear and concise. Claim 12 has therefore not been fully searched.
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

27/07/98

International application No. PCT/SE 98/01368	
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9408585 A1	28/04/94	AT 153855 T AU 674038 B AU 5328694 A DE 69311361 D,T EP 0664707 A,B ES 2105333 T JP 8502485 T US 5480900 A US 5605922 A	15/06/97 05/12/96 09/05/94 08/01/98 02/08/95 16/10/97 19/03/96 02/01/96 25/02/97

US 4132738 A	02/01/79	AU 515772 B AU 4449379 A CA 1191132 A DE 2902699 A,B,C FR 2430403 A,B FR 2473509 A,B GB 2014989 A,B GB 2099814 A,B JP 1194012 C JP 54115351 A JP 58026910 B SE 441673 B,C SE 445109 B,C SE 453830 B,C SE 453990 B,C SE 7813385 A SE 8303910 A SE 8303911 A SE 8303912 A US 4275224 A US 4331688 A US 4415592 A US 4742080 A US 4833157 A	30/04/81 30/08/79 30/07/85 30/08/79 01/02/80 17/07/81 05/09/79 15/12/82 12/03/84 07/09/79 06/06/83 28/10/85 02/06/86 07/03/88 21/03/88 24/08/79 08/07/83 08/07/83 08/07/83 23/06/81 25/05/82 15/11/83 03/05/88 23/05/89

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PATENT COOPERATION TREATY
PCT
INTERNATIONAL PRELIMINARY EXAMINATION REPORT

REC'D	01 OCT 1999
WIPO	PCT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference Pha-1775-PCT	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/SE98/01368	International filing date (day/month/year) 10.07.1998	Priority date (day/month/year) 11.07.1997
International Patent Classification (IPC) or national classification and IPC ₆ A 61 K 31/557		
Applicant Pharmacia & Upjohn AB et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 5 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of _____ sheets.
3. This report contains indications relating to the following items:
 - I Basis of the report
 - II Priority
 - III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
 - IV Lack of unity of invention
 - V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
 - VI Certain documents cited
 - VII Certain defects in the international application
 - VIII Certain observations on the international application

Date of submission of the demand 03.02.1999	Date of completion of this report 02.09.1999
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. 08-667 72 88	Authorized officer Göran Karlsson/Els Telephone No. 08-782 25 00

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/SE98/01368

I. Basis of the report

1. This report has been drawn on the basis of (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

 the international application as originally filed. the description, pages _____, as originally filed,
pages _____, filed with the demand,
pages _____, filed with the letter of _____,
pages _____, filed with the letter of _____ the claims, Nos. _____, as originally filed,
Nos. _____, as amended under Article 19,
Nos. _____, filed with the demand,
Nos. _____, filed with the letter of _____,
Nos. _____, filed with the letter of _____ the drawings, sheets/fig _____, as originally filed,
sheets/fig _____, filed with the demand
sheets/fig _____, filed with the letter of _____
sheets/fig _____, filed with the letter of _____

2. The amendments have resulted in the cancellation of:

 the description, pages _____ the claims, Nos. _____ the drawings, sheets/fig _____

3. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the supplemental Box (Rule 70.2(c)).

4. Additional observations, if necessary:

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/SE98/01368

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

 the entire international application, claims Nos. 6-8, 11-12

because:

 the said international application, or the said claims Nos. 6-11relate to the following subject matter which does not require an international preliminary examination (*specify*):

A method for treatment of the human or animal body by therapy (PCT Rule 67.1 (iv)).

 the description, claims or drawings (*indicate particular elements below*) or said claims Nos. 6-11
are so unclear that no meaningful opinion could be formed (*specify*):

The expression "a selective agonist for EP1 prostanoid receptors" in claim 12 is indefinite. According to PCT Article 6, the claims shall be clear and concise. Claim 12 has therefore not been fully searched.

- the claims, or said claims Nos. _____ are so inadequately supported
by the description that no meaningful opinion could be formed.
- no international search report has been established for said claims Nos. _____

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/SE98/01368

V. Resoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	5	YES
	Claims	1-4, 12	NO
Inventive step (IS)	Claims	5	YES
	Claims		NO
Industrial applicability (IA)	Claims	5	YES
	Claims		NO

2. Citations and explanations

The invention relates to a composition for the treatment of glaucoma and ocular hypertension comprising a therapeutically active and physiologically acceptable amount of a prostaglandin analogue.

The expression "a selective agonist for EP1 prostanoid receptors" in the claims is indefinite. According to Article 6, the claims shall be clear and concise.

Claims 1-4 concern the first medical use of known prostaglandins and are thus lacking novelty, see for example US, A, 4132738.

J. Biol. Chem. 1993, Vol. 268 pp 20175-8 discloses that 17-phenyl trinor PGE2 and sulprostern are EP1 agonists. Natural products reports, Vol. 7, 1990 pp 409-445 further discloses that 16,16-dimethyl-PGE2 and enprostil (RS-84-135) are potent EP1 receptor agonist, see page 436.

WO, A, 9408585 discloses the use of these compounds for the treatment of glaucoma. Claim 12 is therefore lacking novelty.

There is however no information in the prior art that a prostaglandin according to the present claim 5 is useful for the treatment of glaucoma and ocular hypertension. Claim 5 is therefore considered to fulfil the requirements of novelty, inventive step and industrial applicability.

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.

PCT/SE98/01368

Supplemental Box

(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: V

No unified criteria exist in the PCT for assessing the industrial applicability of the subject matter of the presently worded claims. Whether the claims are acceptable may depend on how the claims are formulated. Some countries allow claims to a known compound for a first use in medical treatment (first medical indication). They also allow claims to the use of such a compound for the manufacture of a medicament for a new medical treatment (second medical indication). Furthermore, claims to a known pharmaceutical preparation for use in a new medical treatment are allowed in at least one country.

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INTERNATIONAL SEARCH REPORT

International application No. PCT/SE 98/01368
--

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A61K 31/557

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9408585 A1 (ALCON LABORATORIES, INC.), 28 April 1994 (28.04.94) --	1-3,12
X	Journal of Lipid Mediators, Volume 6, 1993, David F. Woodward et al, "Intraocular pressure effects of selective prostanoid receptor agonists involve different receptor subtypes according to radioligand binding studies" page 545 - page 553 --	1-3,12
A	The Journal of Biological Chemistry, Volume 268, No 27, Sept 1993, Akiko Watabe et al, "Cloning and Expression of cDNA for a Mouse EP1 Subtype of Prostaglandin E Receptor", page 20175 - page 20178 --	12

 Further documents are listed in the continuation of Box C. See patent family annex.

* Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier document but published on or after the international filing date	"Y"	document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means		
"P" document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

12 October 1998

Date of mailing of the international search report

01 -11- 1998

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Swedish Patent Office
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Authorized officer

Göran Karlsson
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 98/01368

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Natural product reports, Volume 7, No 5, 1990, D. E. Bays et al, "Inhibitors of Gastric Acid Secretion", page 409 - page 445, see page 436 --	12
X	US 4132738 A (HAROLD C. KLUENDER ET AL), 2 January 1979 (02.01.79) -----	1-4

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 98/01368

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: 6-8, 11
because they relate to subject matter not required to be searched by this Authority, namely:
A method for treatment of the human or animal body by therapy, see rule 39.1.
2. Claims Nos.: 12
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
The expression "a selective agonist for EP1 prostanoid receptors" in claim 12 is indefinite. According to PCT Article 6, the claims shall be clear and concise. Claim 12 has therefore not been fully searched.
3. Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

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INTERNATIONAL SEARCH REPORT
Information on patent family members

27/07/98

International application No.	
PCT/SE 98/01368	

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO	9408585	A1	28/04/94	AT 153855 T AU 674038 B AU 5328694 A DE 69311361 D,T EP 0664707 A,B ES 2105333 T JP 8502485 T US 5480900 A US 5605922 A	15/06/97 05/12/96 09/05/94 08/01/98 02/08/95 16/10/97 19/03/96 02/01/96 25/02/97
US	4132738	A	02/01/79	AU 515772 B AU 4449379 A CA 1191132 A DE 2902699 A,B,C FR 2430403 A,B FR 2473509 A,B GB 2014989 A,B GB 2099814 A,B JP 1194012 C JP 54115351 A JP 58026910 B SE 441673 B,C SE 445109 B,C SE 453830 B,C SE 453990 B,C SE 7813385 A SE 8303910 A SE 8303911 A SE 8303912 A US 4275224 A US 4331688 A US 4415592 A US 4742080 A US 4833157 A	30/04/81 30/08/79 30/07/85 30/08/79 01/02/80 17/07/81 05/09/79 15/12/82 12/03/84 07/09/79 06/06/83 28/10/85 02/06/86 07/03/88 21/03/88 24/08/79 08/07/83 08/07/83 23/06/81 25/05/82 15/11/83 03/05/88 23/05/89

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